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The Synthesis of Oligodeoxynucleotides Containing 2-Thiothymine and 5-Methyl-4-Pyrimidinone Base Analogues

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Abstract: *A procedure is described for the preparation of two thymidine analogues in which the O^2-carbonyl is deleted (dH^2T) or converted to an O^2-thione (ds^2T). The phosphoramidites of these nucleosides have been synthesized and used to incorporate the analogues site-specifically into oligodeoxynucleotide sequences.*

There is increasing interest in the synthesis of nucleoside analogues and their incorporation into DNA sequences for the study of ligand-DNA[1] and protein-DNA interactions.[2] A variety of nucleoside derivatives have been prepared that result in the deletion, or change in the nature, of the functional groups present on the heterocyclic bases. Such analogues permit the synthesis of oligodeoxynucleotides in which a single functional group at a preselected position has been deleted or otherwise altered. For the pyrimidine nucleosides, the base analogues prepared have generally involved the deletion or alteration of the N^4-amino group, the O^4-carbonyl or the C_5-methyl group. The simplest such "deletion modification" involves excision of the thymine methyl group by the site-specific replacement of thymidine with 2'-deoxyuridine in the DNA sequence of interest.[3] The deletion of the amino or carbonyl functional group at the 4-position of the pyrimidine nucleosides dC and dT can be accomplished with the synthesis and incorporation of the 2-pyrimidinone nucleosides as we[4] and others[5] have described. A series of related reports also describe the preparation of 4-thiopyrimidine derivatives as a route to alter the hydrogen bonding character of the O^4-carbonyl as an alternative to deletion of the functional group.[5,6]

In the present report, we describe the synthesis of oligodeoxynucleotides containing two thymidine analogues in which the O^2-carbonyl has been deleted or converted to the corresponding thione. This has been accomplished by the synthesis of 2-thiothymidine (**3**) and 5-methyl-4-pyrimidinone-2'-deoxyriboside (**5**) and the conversion of both derivatives into the corresponding phosphoramidites **4** and **6**. The synthesis of 2-thiothymidine has been described by Faeber and Scheit[7] using the procedures originally developed by Todd and coworkers.[8] In this pathway, 3'-O-acetylthymidine (**1**) was converted to the anhydro derivative **2** via 5'-deoxy-5'-iodo-3'-O-acetylthymidine (see Scheme, following page). The cyclic anhydro compound **2** was treated with H_2S in dimethylformamide under pressure to produce 2-thiothymidine in 42% yield.[9] Reaction of 2-thiothymidine with 4,4'-dimethoxytrityl chloride[10] followed by β-cyanoethoxydiisopropyl-aminochlorophospine[11] using essentially standard protocols[12] yielded **4**.[13] Ogihara and Mitsunobu have described a procedure for the desulfurization of 2-thiouridines using dipotassium diazenedicarboxylate or a related procedure employing $N_2H_4/H_2O_2/CuSO_4$.[14] These reactions functioned well to desulfurize 2-thiothymidine (**3**) in small quantities under the conditions described. However, in our hands desulfurization with Raney nickel proved to be the most efficient procedure. Raney nickel can be used to desulfurize purines[15] but has not generally resulted in consistent yields in the desulfurization of pyrimidines such as 4-thiopyrimidines.[16] We examined the desulfurization of **3** using

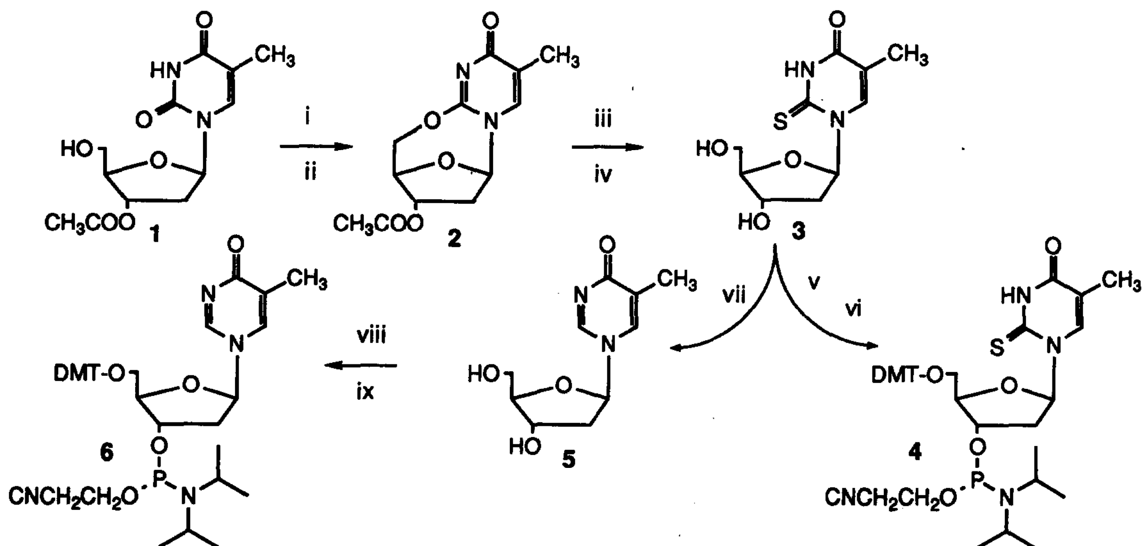

Figure 1. Conversion of 3'-O-acetylthymidine to the phosphoramidite derivatives of ds^2T and dH^2T. Conditions for the reactions were as follows: i) $CH_3P(OC_6H_5)_3I$, dimethylformamide; ii) $CH_3COO^{-+}Ag$, acetonitrile; iii) H_2S, dimethylformamide, Triethylamine, -78 °C; iv) Methanolic ammonia; v) DMT-Cl, pyridine; vi) $[(CH_3)_2CH]_2NP(Cl)OCH_2CH_2CN$, dichloromethane, diisopropylethylamine; vii) Raney Ni, H_2O, 50 °C; viii) DMT-Cl, pyridine; ix) $[(CH_3)_2CH]_2NP(Cl)OCH_2CH_2CN$ dichloromethane, diisopropylethylamine.

Raney nickel and observed that the reaction proceeded rapidly and efficiently to generate the 5-methyl-4-pyrimidinone **5** in a 61% isolated yield.[17] The pyrimidinone could be smoothly converted to the phosphoramidite **6** by reaction with 4,4'-dimethoxytrityl chloride[18] and β-cyanoethoxydiisopropylaminochlorophospine.[19]

We examined the sensitivity of ds^2T (**3**) and dH^2T (**5**) to three sets of conditions commonly experienced during the synthesis of DNA sequences: (i) dichloroacetic acid/dichloroethane, 20 min ambient temperature, (ii) $I_2/THF/H_2O$/lutidine, 30 min ambient temperature, and (iii) 28% aqueous ammonia, 18 h 50 °C. TLC and HPLC analyses of these reaction mixtures indicated that the dH^2T derivative was stable to each of the conditions over the period of time described. Treatment with acid had no effect upon ds^2T, and only ~2% degradation of the nucleoside occurred after 18 h in aqueous ammonia (50 °C). However, ds^2T rapidly decomposed to a variety of products upon treatment with the iodine solution for 30 min. HPLC analysis after 30 min. indicated that only 31% of the remaining material was ds^2T, and a number of species were present including dT.

Both phosphoramidite derivatives **4** and **6** were soluble in anhydrous acetonitrile and could be used to make a series of dodecamers using exactly the same concentrations and coupling conditions typically employed with the "normal" nucleosides. After assembly and deprotection of the oligodeoxynucleotides, they were isolated using procedures described previously.[20] The sequences obtained in this manner containing a single dH^2T residue chromatographed as single species using reversed-phase HPLC columns. By comparison, sequences prepared with a single ds^2T residue typically exhibited three closely eluting peaks with the second peak being the major product. A small quantity of each oligodeoxynucleotide was digested with a mixture of snake venom phosphodiesterase and bacterial alkaline phosphatase to confirm that the base analogues had been incorporated into the sequences. HPLC analysis of the hydrolysate resulting

from the dodecamer d[CGCGAAT(H^{2}T)CGCG] indicated that one equivalent of dH^{2}T was present in the sequence (Figure 2a). The three HPLC analyses of the products resulting from the synthesis of d[CGCGAAT(s^{2}T)CGCG] varied. Neither of the minor products contained significant amounts of the analogue ds^{2}T. The major product had the expected ratios of the common nucleosides and one equivalent of ds^{2}T, but also contained an unidentified residue eluting near 10 min (see "?" Figure 2b).

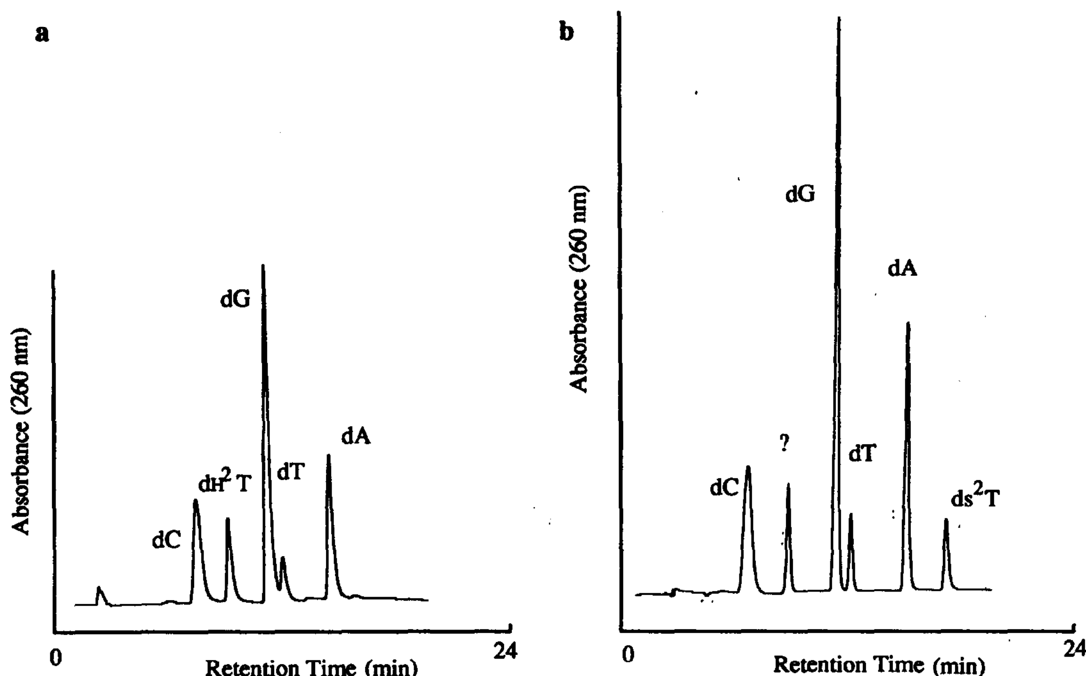

Figure 2. HPLC analysis of the hydrolysis of (a) d[CGCGAAT(H^{2}T)CGCG] and (b) d[CGCGAAT(s^{2}T)CGCG].[21]

The base analogue dH^{2}T could be incorporated site-specifically and efficiently into short oligodeoxynucleotides using standard procedures and this derivative should be valuable for the study of protein and/or ligand binding in the minor groove of DNA sequences. By comparison, ds^{2}T was generally unstable to the oxidation conditions employed in standard DNA synthesis procedures. While it may be possible to prepare short sequences containing this analogue, competing oxidation reactions may become problematic.

ACKNOWLEDGEMENTS

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9 Spectral characteristics of this compound were identical with those described (see reference 7)

10 ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.9 (s, 3H, CH_3), 2.2-2.6 (m, 2H, $H_{2'}$, $H_{2''}$), 3.6 (m, 2H, $H_{5'}H_{5''}$), 3.8 (s, 6H, OCH_3), 4.0 (m, 1H, $H_{4'}$), 4.2 (m, 1H, $H_{3'}$), 5.4 (m, 1H, 3'-OH), 6.8 (t, 1H, $H_{1'}$), 7.0-8.0 (m, 13H, Ar-H), 8.8 (s,1H, H6), 9.4 (brs, 1H, NH). U.V.(methanol) λ_{max} = 272 nm. R_f (dichloromethane/methanol, 95/5) = 0.51

11 ^{31}P NMR ($CDCl_3$) δ = 148.8, 149.1. R_f (dichloromethane/methanol, 95/5, trace TEA) = 0.75.

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17 ^{1}H NMR (DMSO-d_6, 300 MHz) δ = 1.8 (s, 3H, CH_3), 2.0-2.2 (m, 2H, $H_{2'}$,$H_{2''}$), 3.4 (m, 2H, $H_{5'}$,$H_{5''}$), 3.8 (m, 1H, $H_{4'}$), 4.2 (m, 1H, $H_{3'}$), 5.0 (t, 1H, J = 5.0 Hz, 5'-OH), 5.4 (d, 1H, J = 3.5 Hz, 3'-OH), 5.8 (t, 1H, J = 6.8 Hz, $H_{1'}$), 7.8 (s, 1H, H6), 8.4 (s, 1H, H2). UV (methanol) λ_{max} = 244 nm.
R_f (dichloromethane/methanol, 4/1) = 0.26.

18 ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.6 (s, 3H, CH_3), 2.2-2.4 (m, 2H, $H_{2'}$,$H_{2''}$), 3.6 (m, 2H, $H_{5'}$,$H_{5''}$), 3.8 (s, 6H, OCH_3), 4.0 (m, 1H, $H_{4'}$), 4.4 (m, 1H, $H_{3'}$), 5.4 (m, 1H, 3'-OH), 5.9 (t, 1H, $H_{1'}$), 6.8-7.4 (m, 13H, Ar-H), 7.8 (s, 1H, H6), 8.4 (s, 1H, H2). U.V.(methanol) λ_{max} = 231 nm. R_f (dichloromethane/methanol, 9/1) = 0.50.

19 ^{31}P NMR ($CDCl_3$) δ = 151.6, 151.9. R_f (dichloromethane/methanol, 95/5 trace TEA) = 0.65

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